Mucosal Infection with *Neisseria gonorrhoeae*

Bacterial Adaptation and Mucosal Defenses

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Introduction and overview

Neisseria gonorrhoeae is a pathogen responsible for a number of common and important disease syndromes (1). The organism is transmitted as a mucosal infection (2); humans are the only definitive host. Indeed, it has been difficult, if not impossible, to establish a relevant infection in other animals, including several primate species (3).

The correlation between gonococcal disease and the biology of the organism has been studied extensively, and represents the purpose of this review. The most critical interactions occur at the mucosal surface. The organism must attach to epithelial cells and multiply. The organism must resist any innate host defenses, as well as defenses that may have evolved from prior infection. In male hosts, urethral infection with most strains of *N. gonorrhoeae* leads to symptomatic exudative inflammatory response (1). However, infection of the endocervix and other mucous membranes may be less obvious. In addition, some strains of gonococci may remain in the urethra or endocervix for long periods of time without producing symptoms (2). The precise reasons for "quiescent infection" are not known, but such gonococcal strains generally have unique nutritional requirements, extreme sensitivity to antibiotics, and limited ability to activate the complement cascade or to generate neutrophil chemoattractant factors (2).

Gonococcal disease is important because it is a common cause of salpingitis and female infertility (4). Under conditions that are still not well understood, the organism may leave mucous membranes (such as the endocervix) and infect the epithelial cells of the fallopian tubes. Such migration requires penetration of several immunological and nutritional barriers, and a transport "carrier," since gonococci are not motile. Either refluxed menstrual blood or attachment to spermatozoa may aid in transit upstream against ciliated fallopian tube cells.

Gonococci less commonly produce bacteremia and systemic disease. Disseminated gonococcal infection (DGI) includes infection of the skin, synovium, and joints. Rarely, meningitis or endocarditis may occur. To produce DGI, gonococci must evade serum defenses and phagocyte surveillance.

In general, it is believed that a wide spectrum of gonococcal disease is possible because of multiple sophisticated adaptations of gonococci to selective pressures of the host. In this article we will review our current understanding of the pathogenesis of gonococcal infection at the mucosal surface. We hope to convey exciting advances made possible through the tools of molecular biology and more recent human experimentation.

Gonococcal biology represents a model by which to understand the effects of selective pressure in vivo and a myriad of adaptive microbial strategies.

Gonococcal aim 1: establishing infection

Attachment (Fig. 1). The critical first step in establishing infection is attachment to host cells. Attachment of *N. gonorrhoeae* to epithelial cells has been studied extensively (5). Thirty years ago Kellogg and his colleagues demonstrated that only gonococci expressing pili (or a factor coexpressed with pili) were able to establish infection in male human volunteers (6). This result has recently been validated by Boslego and co-workers (P. Hitchcock, personal communication). There are several other nonpilus surface ligands for attachment, including opacity (Opa)[1] proteins (7), a 37-kD protein that binds to certain cellular glycolipids (8). Multiple different adhesins may be required to effect attachment to different types of cells in diverse environments such as the urethra, cervix, fallopian tubes, and/or rectum. Evidence for this conjecture comes from a variety of in vitro studies, and from the very rapid selection in vivo of new types of pili (9) and Opa proteins (10), before a specific immune response. Both pili and Opa proteins are clearly important in vivo; the importance of other adhesins remains to be established.

Opa proteins are interesting from several points of view. They are members of a set of as many as 11 closely related outer membrane proteins, most of which confer an opaque appearance to colonies. The colonial appearance is due to increased adherence between gonococcal cells (11) which form multicellular clumps (infectious units), potentially important in initiation or maintenance of gonococcal infection. Opa proteins also appear to directly increase binding to some cells (7). Only a few (or none) of the Opa proteins are expressed at one time. Variations or "switching" between different Opa proteins occurs at a rate of about 10^{-3} per cell per generation. Control of Opa expression occurs by means of variations in length of a five-nucleotide DNA repeat $(CTCTT)_n$ within the signal sequence-encoding region of Opa genes; variations in numbers of the $(CTCTT)_n$ repeat result in translational frame shifts, and there-

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1. *Abbreviations used in this paper:* CMP-NANA, cytidine monophosphate-*N*-acetylneuraminic acid; DGI, disseminated gonococcal infection; LF, lactoferrin; LOS, lipooligosaccharide; Opa, opacity; Por, the gonococcal porin; Rmp, reduction modifiable protein.

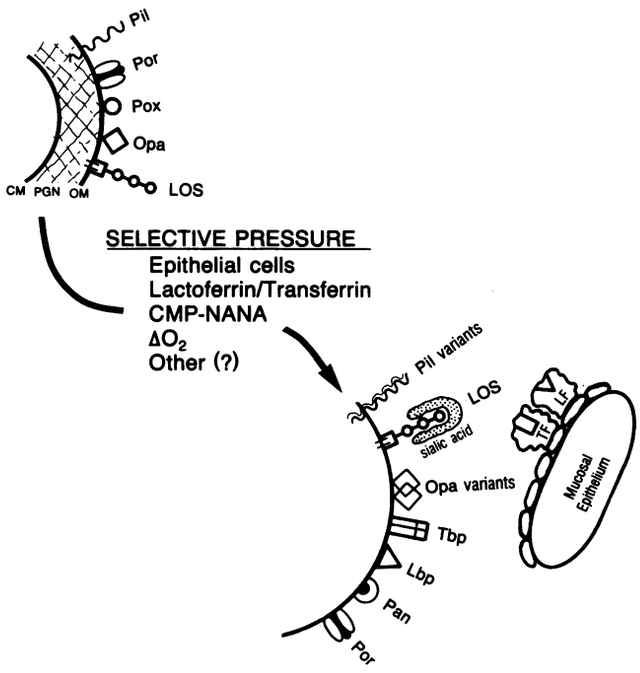

Figure 1. Gonococcal attachment and adaptation. Gonococci demonstrate constitutive expression of several outer membrane (OM) structures during growth in vitro or in vivo, including porin protein (Por), Protein III (reduction modifiable protein, Rmp), and the core of LOS. Selective pressure through exposure to a mucosal environment may lead to substantive changes in the outer membrane, including expression of new variants of pili (Pil), Protein II (opacity protein, Opa), and LOS polysaccharides. Moreover, genes that are not transcribed under usual growth conditions in vitro may be transcribed in vivo, leading to formation of iron-repressible proteins for binding transferrin (Tbp) or lactoferrin (Lbp), or anaerobically expressed proteins (Pan). Certain oxygen-regulated proteins (Pox) are expressed only under aerobic conditions. It is possible that transferrin (TF) and/or lactoferrin (LF) associated with epithelial cells facilitates attachment of gonococci through Tbp or Lbp. Growth of gonococci in vivo leads to sialylation of LOS with host-derived cytidine monophosphate-*N*-acetyl neuraminic acid (CMP-NANA). CM, cytoplasmic membrane; PGN, peptidoglycan.

fore control of expression at the level of translation (12, 13). In contrast, similarly high frequencies of phase and antigenic variations of pili are due to recombination between incomplete, variant "silent" pilin DNA cassettes and pilin structural genes, and other transcriptional control mechanisms (14–16). High frequency antigenic variation serves the dual purposes of escape from immune surveillance, and provision of specific ligands for different cell receptors.

Epithelial cell invasion. Several investigators have demonstrated gonococcal invasion of epithelial cells in vitro (17, 18). This is believed to represent parasite-directed endocytosis (19). However, it is not clear whether epithelial invasion represents a "normal" part of uncomplicated mucosal infection, or is important only to deeper, complicated infections, such as salpingitis or the bacteremia–arthritis syndrome. Certain outer membrane proteins may facilitate invasion of epithelial cells, including outer membrane protein I (Por, the gonococcal porin) (20), and some, but not all, Opa proteins (17, 18).

Adaptation (Fig. 1). Gonococci rapidly adapt to their microenvironment in vivo (9, 10), and similar adaptation can be demonstrated quite easily in vitro (21–23). Variations in pili and Opa are primarily genotypic (i.e., variations within the *pil* or *opa* genes that alter their expression). Other important variations are primarily phenotypic (i.e., change in expression of genes, without alteration in structure of the respective genes). These occur principally in response to environmental stresses, probably due to signal transduction mechanisms that alter expression of particular genes. Examples include increased synthesis of iron-repressed outer membrane proteins (Frps), some of which are necessary for specific gonococcal receptors for binding human iron transport proteins such as transferrin and lactoferrin (21); synthesis of outer membrane proteins that normally are repressed by oxygen (Pan; 22); synthesis of growth-related stress proteins (GSPs; 24), and synthesis of proteins evoked by contact with epithelial cells (25). Compelling evidence for expression in vivo of some of these stress proteins can be derived from experiments in which serum from subjects with uncomplicated gonococcal infection, pelvic inflammatory disease, and/or disseminated gonococcal infection demonstrate antibodies to one or more of these proteins (reviewed in reference 26). These data indicate that gonococci sometimes grow in vivo under conditions of relative iron deprivation and anaerobiosis.

It is possible that different outer membrane proteins are formed in different sites of disease, such as the urethra, endocervix, and fallopian tube, although this has not been well studied. Recognition that in vitro phenotypes may be considerably different than those expressed in vivo has critical implications for vaccine development.

Indeed, these observations are so important that they have lead to fairly extensive experimentation with human volunteer subjects (9, 10, and unpublished data, A. Jerse, J. Cannon, and M. S. Cohen). Particular Opa proteins (10) or an increased number of Opa proteins (unpublished data) appear to be selected in vivo. Variations of gonococcal lipooligosaccharide (LOS) also seem to be important (27). Schneider and coworkers studied LOS variation in volunteer subjects challenged with intraurethral *N. gonorrhoeae* (27), and demonstrated an evolution of LOS phenotype during the course of infection. This evolution in part reflects sialyiation of LOS by cytidine monophosphate-*N*-acetylneuraminic acid (CMP-NANA), a change that provides gonococci with an unstable form of serum resistance that may be required for infection (see below). It also reflects high frequency ($\sim 10^{-3}$) variation in expression of certain core sugars in LOS (23). The mechanisms for variable expression of LOS core sugars are unknown.

A schema of gonococcal adaptations that aid in establishing infection is provided in Fig. 1. Adherence ligands such as pili and one or more Opa proteins allow initial attachment of the bacteria to epithelial cells. Selective pressure leads to expression of a variety of other proteins potentially important for more effective attachment, nutritional support, cellular invasion, replication, and perhaps, most importantly, evasion of human host defenses.

Gonococcocl aim 2: evasion of mucosal defenses

Inflammation associated with gonococcal infection represents a combination of failed host defense mechanisms and the direct cytotoxicity of the organism. Mucosal secretions contain a variety of nonspecific host defenses including lysozyme and iron binding proteins such as transferrin and lactoferrin (28). Most gonococci are well adapted to use these nonspecific de-

fenses to their own advantage. Specific host defenses are believed to play a more critical role in the inflammatory biology of gonococcal infection. These include the interaction of gonococci with serum and antibodies, neutrophilic phagocytes, and the cells and cytokines required for cell-mediated immunity.

Gonococci, serum, and humoral immunity (Fig. 2). Human serum, even in subjects who have not had prior infection with *N. gonorrhoeae,* contains a variety of antibodies bactericidal for many gonococci (29–31, and reviewed in 26). These antibodies are generally directed against one or more components of LOS, but they may also be directed against a variety of other antigens (29–31). Subjects with prior gonococcal infection demonstrate antibodies to multiple antigens, both in serum and mucosal secretions (29–31).

How do gonococci resist serum-mediated death? Twenty years of study have lead to an exciting but highly complex answer to this question. After growth in vitro, some gonococci are phenotypically sensitive to complement-mediated killing by human sera, whereas others are stably resistant to the same sera. It now seems likely, however, that most gonococci are phenotypically resistant to killing by normal human serum in vivo. Genetically determined stable serum resistance is probably due to the particular type of Por protein expressed (32); these organisms are often the cause of disseminated gonococcal disease. Other strains that become sensitive to serum only after growth in vitro rapidly acquire phenotypic serum resistance through exposure to human red and white blood cells (33), serum (34), or growth in vivo (27, 35). Phenotypic conversion to serum resistance in vivo is due to sialylation of LOS by gonococcal use of host-derived CMP-NANA (27, 35). CMP-NANA is the substrate used by mammalian cells for sialylation

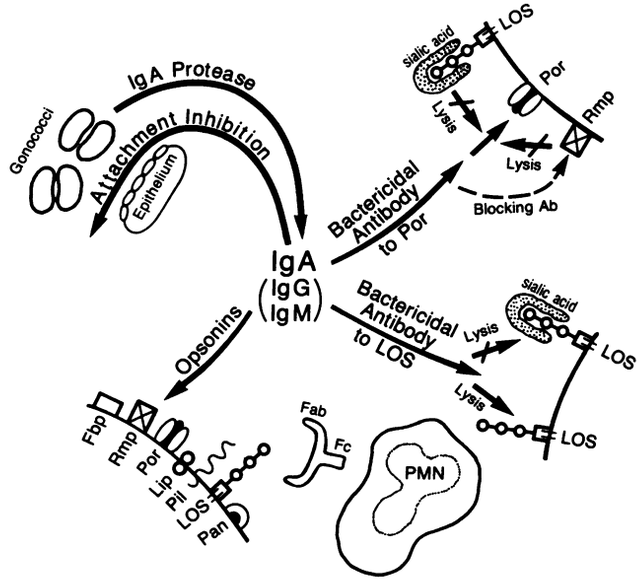

Figure 2. Gonococci resist serum-medicated defenses. Secretory IgA may block gonococcal attachment, but a gonococcal protease degrades IgA_1. IgG and IgM directed against porin (Por) and/or LOS lead to complement-mediated gonococcal lysis. However, blocking antibodies directed against Protein III (Rmp) or sialylation of LOS may inhibit serum killing, most likely by interfering with complement fixation (37, 38). IgG and IgM could also opsonize gonococci and facilitate their uptake by phagocytes. Sialylation of LOS may interfere with opsonization (40).

of glycolipids and glycosphingolipids. During gonococcal infection, LOS epitopes capable of sialylation may be preferentially expressed (27, 36), and most or all gonococci produce the enzyme sialyltransferase required to effect this modification to LOS structure.

Another distinct mechanism for phenotypic serum resistance is binding of blocking antibodies to outer membrane protein III (Rmp) that interfere with natural or acquired bactericidal antibodies directed against outer membrane Por or LOS (37, 38). Gonococcal Rmp is similar in structure to *Escherichia coli* ompA (37) and to analagous proteins in other *Neisseriae,* including meningococci, and it is likely that antibodies formed to other bacteria in normal hosts may be important in the genesis of cross-reactive anti-gonococcal blocking antibodies. Blocking antibodies have been demonstrated in sex workers with repeated gonococcal infection (39), supporting the idea that formation of blocking antibody may enhance susceptibility to mucosal infection.

The multiplicity of mechanisms employed by gonococci to protect against damage by antibody and complement suggest the importance of this defense. The mechanisms for protection against complement involve inability of the terminal membrane attack complex (5b-9) to insert into the bacterial membrane in the configuration required for killing (reviewed in 38). Serum sensitive or resistant gonococci bind similar concentrations of C7 and C9 molecules from normal human serum (38). However, the binding patterns are different between serum resistant and sensitive organisms (38). Sialylation may limit or alter complement binding, thereby interfering with killing of gonococci by LOS antibodies, and/or antibodies directed against Por, as well as complement-dependent phagocytosis (see below, 40). Complement components could also play a direct role in injury to host cells, but there is no evidence for this in gonococcal infection.

Interaction between gonococci and serum leads to formation of chemoattractant factors that are expected to evoke neutrophilic inflammation. Densen and co-workers (41) showed that serum-sensitive gonococci (which are the usual cause of symptomatic urethral infection) generate complement-derived chemoattractants at a faster rate and in higher concentration than phenotypically stable serum-resistant isolates. Stable serum-resistant gonococcal isolates bind iC3b in vivo and in vitro better than serum-sensitive strains, allowing inactivation of C3 cleavage products and preventing full expression of C5 convertase required to generate the chemotaxin C5a (42). Sialylation of LOS does not appear to effect C3b cleavage (42). Genotypically stable, serum-resistant isolates are associated with asymptomatic male urethral infection (43), perhaps due to their relative inability to generate chemotaxins and a local purulent response (41–43).

Mucosal antibodies and secretory IgA. IgG, IgM, and IgA antibodies directed against gonococcal antigens have been detected at mucosal surfaces (29, and reviewed in 26). IgA is made daily in concentrations higher than other antibody classes, and two unique secretory subclasses ($SIgA_1$ and $_2$) are generated by mucosal lymphocytes (reviewed in 28). The precise function of SIgA is not known, but it is clearly not opsonic. Tramont and co-workers demonstrated that both IgG and IgA can prevent attachment of gonococci to buccal epithelial cells (44). However, gonococci (and other pathogenic Neisseria) form an IgA_1 protease that cleaves the hinge region of the IgA

dimer. The ability of SIgA to prevent gonococcal infection in vivo has not been demonstrated.

Phagocytic cells (Fig. 3)

Gonococci are pyogenic bacteria. Although the prevalence of asymptomatic or oligosymptomatic gonococcal strains helps to explain the persistence of this disease in humans (1), the majority of subjects with acute gonococcal infection develop a frank and painful purulent exudate (1, 2). Granulocytes evoked in this setting probably are responsible for the majority of mucosal damage observed. Interactions between *N. gonorrhoeae* and phagocytic cells have been studied extensively. Several important questions have not been answered, including whether phagocytes are capable of killing all gonococci in a small inoculum, and whether gonococcal adaptation is required for survival during phagocyte attack.

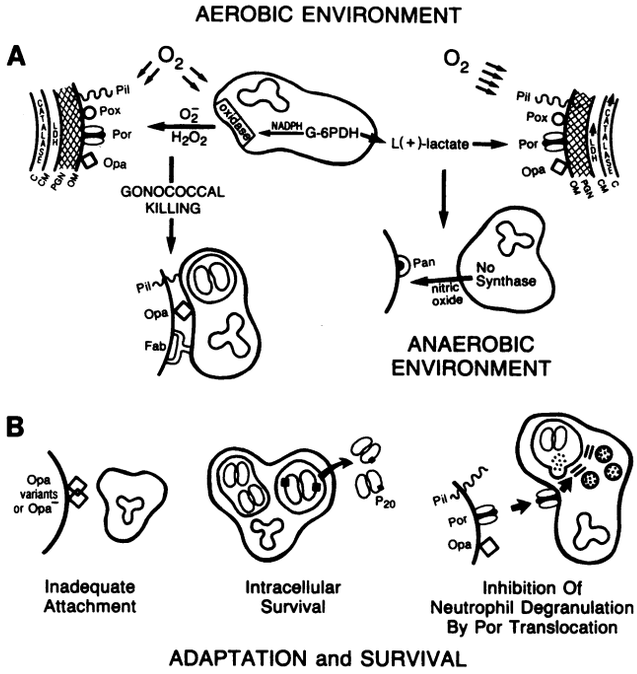

Figure 3. Gonococci and phagocytes. (*A*) Phagocytes generate O_2^-, H_2O_2, and other reactive oxygen intermediates, which (in concert with microbicidal proteins) might be expected to kill gonococci. However, gonococcal adaptation (Fig. 3, *a* and *b*) is likely an important part of resistance in vivo. Gonococci exposed to neutrophils demonstrate an increase in catalase, which facilitates resistance to neutrophils. Gonococcal use of neutrophil L(+)-lactate allows O_2 consumption adequate to inhibit neutrophil generation of O_2^-, and could ultimately lead to an anaerobic environment. Neutrophil-derived nitric oxide could replace O_2 as a terminal electron acceptor, required for bacterial growth. C, cytoplasm; CM, cytoplasmic membrane; PGN, peptidoglycan; OM, outer membrane; Pil, pilin; Por, porin; Opa, opacity proteins; G-GPDH, glucose 6-phosphate dehydrogenase; LDH, lactate dehydrogenase; NO, nitric oxide. (*B*) Several other mechanisms accounting for the survival of gonococci during neutrophil attack have been proposed. Inadequate attachment of Opa^- gonococci and/or gonococci expressing variant Opa proteins could lead to extracellular survival; pili and/or other gonococcal "factors" could also inhibit phagocytosis directly. Formation of a unique 20-kD protein after phagocytosis could permit intracellular survival. Translocation of gonococcal Por protein to the phagocyte could inhibit neutrophil deregulation, also leading to intracellular survival of gonococci.

Phagocytes employ different microbicidal mechanisms for aerobic and anaerobic conditions. Both aerobic and anaerobic organisms may be recovered from vaginal secretions (45) and the male urethra (46). Gonococci are facultative anaerobes (47), and Clark and co-workers demonstrated serum immune responses during urethral infection to Pan (anaerobic) gonococcal proteins (48). It can be assumed that in some phase of gonococcal infection the mucosa is aerobic, if only at the male anterior urethra. During this time, gonococci would use oxygen as a terminal electron acceptor and mucosal substrates for growth. Iron could be provided by lactoferrin, transferrin, or other mucosal iron-binding proteins. Gonococci preferentially use L(+)-lactate for growth rather than glucose (49). Lactate is found in serum and is generated by phagocytes during aerobic glycolysis evoked by phagocytosis (50). The use of L(+)-lactate by gonococci is potentially important because of phenotypic changes that may occur through the use of this substrate (49), and because gonococci using L(+)-lactate demonstrate markedly accelerated competition for molecular oxygen (50). Indeed, through competition for molecular oxygen gonococci may prevent neutrophil generation of oxidants (see below, and Fig. 3), and help to generate a strict anaerobic environment, thereby potentiating growth of obligate anaerobes (e.g., Bacteroides species) recovered from the fallopian tubes of women with salpingitis (4). Gonococci adapt to anaerobic conditions through the use of nitrite as a terminal electron acceptor (22, 47, 48). Nitrite can be recovered from several tissue sites and is generated by neutrophils and macrophages during phagocytosis (51).

Phagocytosis. Phagocytic cells unquestionably come into intimate contact with gonococci. Examination of urethral neutrophilic exudates demonstrates both intracellular and extracellular gonococci (35). By what mechanisms do gonococci attach to neutrophils? Under what circumstances are neutrophils able to ingest gonococci? A variety of antibodies that react with gonococcal outer membrane proteins have been identified. Some of these antibodies appear to enhance the association of gonococci with neutrophils (52, reviewed in 53). However, most studies fail to differentiate between antibody-enhanced cell association and phagocytosis. Interpretation of the role of "opsonic" antibodies is made more complicated because it has become clear that one or more gonococcal outer membrane proteins also mediate association with leukocytes. Swanson demonstrated that some Opa^+ colonies adhere to granulocytes (54), and using other techniques these results have been verified in several laboratories (55, 56). It has also been demonstrated that certain Opa^+ gonococci are killed by human neutrophils in vitro, whereas Opa^- organisms survive (56). These results imply that Opa^- strains might be selected in vivo. However, only Opa^+ gonococci are recovered from human volunteers inoculated with Opa^- organisms (10, A. Jerse, J. Cannon, M. Cohen, unpublished data), which suggests that expression of Opa proteins is required for establishment of infection, possibly because Opa proteins enhance attachment to and/or penetration of mucosal epithelial cells (17, 18). Further complicating interpretation of results are the ideas that (*a*) gonococci express one or more antiphagocytic factors, perhaps including pili (57); and (*b*) sialylation of LOS may effect opsonization (40) and/or the function of Opa proteins.

Oxygen-dependent events. The enzymatic system that allows phagocytes to reduce molecular oxygen to superoxide anion has been reviewed in detail (58), as have the various oxidants formed by this system (59). Gonococci are sensitive to

superoxide and hydrogen peroxide in vitro (60, 61). Gonococci generate high concentrations of catalase (62, 63), which catalyzes formation of O_2 and H_2O from H_2O_2, but no superoxide dismutase (62–64). A catalase-deficient mutant of gonococci is significantly more sensitive to neutrophils than catalase-replete strains (63). In addition, brief exposure of gonococci to phagocytizing neutrophils or exogenous hydrogen peroxide leads to a significant increase in catalase concentration, as well as resistance to neutrophil attack (63). It seems possible that early upregulation of catalase production may help gonococci survive attack by neutrophils in an aerobic environment.

An additional aspect of the mucosal biology of neutrophils involves the iron-binding protein lactoferrin (LF). Most gonococci express receptors for binding LF, and can use LF as a sole source of iron (21). During phagocytosis, neutrophils secrete LF into the supernatant, which paradoxically may stimulate growth of gonococci by providing a usable source of Fe^{3+}, even at very low levels (i.e., 2%) of iron saturation (65). Fe^{3+} bound to neutrophil LF will not catalyze the formation of hydroxyl radical, which would be formed through the interaction of O_2^- and H_2O_2 in the presence of Fe^{3+} (66). Since hydroxyl radical degrades mucus proteins (67) and damages normal epithelial cells, neutrophil secretion of LF may help to prevent tissue damage.

Anaerobic conditions. Gonococci can also be killed by neutrophils under anaerobic conditions. Proteins that might contribute to such killing have been described in detail, and include cationic proteins of molecular weight of 37,000, 57,000, and cathepsin G (68). Small molecular weight defensins do not appear to play an important role in gonococcal killing.

Surviving neutrophil attack (Fig. 3 *B*). Gonococci clearly survive exposure to neutrophils in vivo. As discussed above, lack of association with neutrophils of Opa^- gonococci, or selected Opa variants, may allow bacterial survival. Upregulation of gonococcal catalase production helps to defend against oxidative killing (63), as does aggressive competition for molecular O_2 (50). Low level penicillin-resistant isolates containing altered penicillin-binding protein 2 demonstrate increased resistance in vitro to killing by cathepsin G (69). A 20-kD gonococcal outer membrane protein appears to inhibit neutrophil intracellular killing (70), and other work suggests a similar role for the gonococcal Por protein (71). In vitro, Por can transfer into mammalian cell lipid bilayers (72); purified Por protein can inhibit neutrophil degranulation, perhaps through interference with neutrophil signal transduction (73). However, all mechanisms to explain gonococcal survival during neutrophil attack are speculative; convincing evidence for the importance of these gonococcal defenses in vivo is lacking.

Cell-mediated immunity and tissue injury

An important aspect of the inflammatory response is the contribution of cell-mediated immunity, as characterized by an interaction between lymphocytes and macrophages, and the generation of a variety of lymphokines and monokines. A systemic lymphocyte response during gonococcal infection has been demonstrated, but does not confer immunity (74). Human peripheral blood lymphocytes demonstrate both antibody-dependent cytotoxicity and natural killing activity directed against *N. gonorrhoeae* (74, 75). Similar activity has been demonstrated in cells isolated from human fallopian tubes (74, 75). Using human fallopian tubes in tissue culture, McGee and co-workers showed that gonococci cause remarkable ciliary inhibition and sloughing, especially compared with nonpathogenic *Neisseriae* (19, 76); gonococcal LOS is at least in part responsible for cytotoxicity observed (19, 75, 76).

Gonococcal antigens are processed by fallopian tube lymphocytes (74), leading to increased secretion of IgA (77) and a variety of cytokines (78). McGee and co-workers reported formation of tumor necrosis factor (TNF) in fallopian tubes, which could also be induced by gonococcal LOS (79). TNF alone may be sufficient to produce the fallopian tube injury caused by gonococcal LOS (79). These results imply that TNF and other cytokines mediate the cytotoxicity of gonococcal infection. These observations have important implications for our understanding of the pathogenesis of pelvic inflammatory disease (PID), and for possible pharmacologic strategies to limit fallopian tube damage during gonococcal infection.

Mucosal inflammation and vaccine development

Most studies of *N. gonorrhoeae* are geared toward a better understanding of the pathogenesis of the disease and/or vaccine development. We have summarized only a small portion of an explosion of information about this organism. Successful vaccine development will require one or more of the following components: (*a*) generation of serum or mucosal antibodies that either block attachment, facilitate complement-mediated lysis of the organism, enhance neutrophilic phagocytosis and/or microbial killing, or mediate antibody dependent cytotoxicity; and (*b*) evocation of cell mediated defenses that prevent infection or ameliorate fallopian tube damage.

Vaccines using pili (80) and Por (personal communication, E. W. Hook, III) failed to confer clinically useful immunity to gonococcal infection. In the case of the pilus vaccine, significant immunity was noted for the homologous strain, but not for heterologous strains expressing different antigenic types of pili (81). Studies with Por vaccine probably were complicated by contamination with blocking antigen (Rmp) that may have interfered with vaccine efficacy (37). Since these studies were performed in the early 1980s, before much was known about either gonococcal phenotypic variation in vivo or about the structure and function of a number of other surface antigens, it is possible that renewed attempts to make a vaccine against relatively stable surface antigens will prove successful. For instance, vaccination with Por purified from recombinant *E. coli* (82) or from gonococcal mutants lacking Rmp (83) should circumvent the problem of blocking antibodies (C. Elkins and P. F. Sparling, unpublished data). Existing data are encouraging in certain respects. Patients developing mucosal infection with gonococci expressing a particular Por serovar may be less likely to develop recurrent infection with strains expressing the same Por (84), suggesting partial mucosal immunity based on Por epitopes. Since Por is not subject to high frequency antigenic variations, identification of conserved immunogenic domains on Por may lead to vaccines based on Por. Other gonococcal surface antigens including some stress proteins are at least partially conserved, and may represent viable vaccine candidates.

Each of these advances represents a clear outgrowth of basic and applied research with *N. gonorrhoeae.* Development of a vaccine, coupled with educational strategies that lead to reductions in risky sexual behaviors (85), could dramatically reduce the consequences of gonococcal infection. Although gonococci are masters of deceit and immune evasion (Table I), development of a vaccine remains an attractive goal.

Table I. Strategies Employed by Gonococci to Escape Immune Surveillance and/or to Adapt Rapidly to Particular Environmental Niches

1. High frequency (10^{-3}) phase and antigenic variation of surface antigens
   a. Pili
   b. Opacity (Opa) proteins
   c. LOS core sugars
2. Gene regulation by environmental factors
   a. Iron-repressible proteins (Frps)
   b. Anaerobically-expressed proteins (Pans)
   c. Oxygen-expressed proteins (Poxs)
   d. Other stress proteins
   e. Catalase
   f. Lactate dehydrogenase (?)
3. Molecular masking
   a. Sialylation of LOS
4. Binding of non-complement fixing "blocking" antibodies
   a. Rmp (protein III)
5. Inactivation of mucosal antibodies
   a. IgA1 protease secretion
6. Subversion of nonspecific host defenses
   a. Specific receptors for transferrin, lactoferrin

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